Drugs Against Cancer: Stories of Discovery and the Quest for a Cure

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CHAPTER 12

Anticancer Drugs that Block Cells in Mitosis

This chapter is about anticancer drugs that were discovered as toxins in certain plants or sea creatures and that were found to block the microtubules that pull the chromosomes apart during mitosis. Microtubules also convey essential molecules down the axons of nerve cells, which is why these same drugs can damage nerve cells.

Anti-cancer drugs from natural products

The natural world of animals, plants, and microorganisms is full of biological warfare agents in the conflicts between various species. Natural poisons serve to ward off predators and competitors. Some were used by people over the ages, both to poison and to cure. A few became very useful as medicines for treatment of cancer (Cragg and Newman, 2004; Vindya et al., 2015). During mitosis, the newly formed chromosome pairs are pulled apart by fibers, called microtubules. Each daughter cell then gets one of the newly formed chromosome pairs. In cancer cells, however, mitosis is often abnormal, and the daughter cells often acquire an abnormal set of chromosomes.

Mitotic inhibitor drugs bind to microtubules and block their functions. I will tell the stories of two classes of these drugs that became important in anticancer therapy, as well as of a class of more recently discovered mitotic inhibitors.

Drugs derived from plants and animals in nature often have more complicated chemical structures than what chemists can easily synthesize in the laboratory. Although making those complicated compounds artificially in the laboratory can be

challenging, living creatures have enzymes that put together surprisingly complicated structures from simple building blocks using enzymes that occur naturally in the cells.

Moreover, evolution provided enormous opportunity for selection of compounds that would help a species to survive and beat off the competition. That is why useful medicines can be derived from nature that our chemists would not be likely to discover. These drugs however evolved as poisons, and so it is not surprising that they have toxic side-effects in patients. But why they have anticancer activity is not entirely clear. This chapter is about the discovery, mechanism of action, therapeutic opportunities and toxic limitations of some of those complicated drug structures

Many anticancer drugs act by damaging DNA or blocking its synthesis, but the mitotic inhibitors do not act on DNA; rather, they interfere with the process of cell division itself. They impair the function of the mitotic spindle, made up of microtubules that normally assure that each daughter cell gets precisely one pair of the newly made chromosomes. When the mitotic spindle is perturbed by these drugs, the cell cannot divide normally and often dies.

The first two classes of mitotic inhibitors to be discovered and developed for cancer treatment were the vinca alkaloids and the taxanes. Their story follows.

The story of Vinca and the periwinkle.

The renowned Madagascar periwinkle (*Vinca rosea*, also known as *Catharanthus roseus*) is a colorful flowering plant with a venerable provenance (Figure 12.1):

"In *The Boke of Secretes of Albartus Magnus of the Vertues of Herbs, Stones and certaine Beastes*, we find: 'Perwynke when it is beate unto pouder with worms of ye earth wrapped about it and with an herbe called houslyke, it induceth love between man and wyfe if it bee used in their meales . . . if the sayde confection be put in the fyre it shall be turned anone unto blue coloure'" (from Botanical.com, A Modern Herbal). Chaucer referred to it as the 'fresh Pervinke rich of hew.'

The periwinkle has world-wide medicinal traditions: "In India, they treated wasp sting with the juice from the leaves. In Hawaii they prescribed an extract of the boiled plant to arrest bleeding. In Central America and parts of South America, they made a gargle to ease sore throat and chest ailments and laryngitis. In Cuba, Puerto Rico, Jamaica and other islands, an extract of the flower was commonly administered as an eyewash for the eyes of infants. In Africa, leaves are used for menorrhagia and rheumatism. Surinamese boil ten leaves and ten flowers together for diabetes. Bahamians take flower decoction for asthma and flatulence, and the entire plant for tuberculosis. In Mauritius, the leaves infusion is given for dyspepsia and indigestion. In Vietnam, it is taken for diabetes and malaria. Curacao and Bermuda natives take the plant for high blood pressure. Indochinese use the stalks

and leaves for dysmenorrhea." (From J. A. Duke, Handbook of Medicinal Herbs, 1985; Magic and Medicine of Plants, 1993; cited by the National Tropical Botanical Garden.)

Obviously, the periwinkle had worldwide reputations for medicinal use for treatment of many ailments. Therefore, it most likely was doing something useful in the sick body – but what, exactly? Among all of those cited uses there is no mention of cancer!

Vinca rosea

Figure 12.1. The Madagascar periwinkle (*Vinca rosea,* also known as *Catharanthus roseus*), the source of vinblastine and vincristine.

Road to Discovery: from the periwinkle to an anticancer drug.

We transition now from ancient lore and tradition to scientific knowledge and medical application. The fascinating history of the discovery of anti-cancer ingredients in the Madagascar periwinkle was summarized in 1958 by Noble (Noble et al., 1958) and in 1968 by Johnson (Johnson, 1968). As early as 1910, Theodore Pickolt, a naturalist and pharmacist, had already described the medicinal use of the periwinkle in Brazil (Johnson et al., 1960). However, the story leading to anticancer drugs from the periwinkle began in a surprising way in 1949, when J. H. Cutts at the University of Western Ontario in Canada learned that in the West Indies a tea made from the leaves was used as a remedy for diabetes (Noble et al., 1958). When investigated in diabetic rats, however, there was no trace of any effect of the tea on diabetes. Undeterred, Cutts tried administering a stronger dose by injecting it instead of just giving it to the rats to sip. To his surprise, the tea-injected rats died within a week.

The rats were found to be dying of infection, which was in turn caused by marked loss of infection-fighting white blood cells. Depletion of white blood cells was

evident not only in the blood stream, but also in the bone marrow where these blood cells are made (Figure 12.2). Cutts may have known that leukocyte or lymph node depletion in mustard gas-exposed sailors a few years earlier was associated with the discovery of the anti-cancer activity of nitrogen mustard (see Chapter 1). Hence, it made sense to purify the white-count-suppressing ingredients from the periwinkle extracts and to test them against cancer.

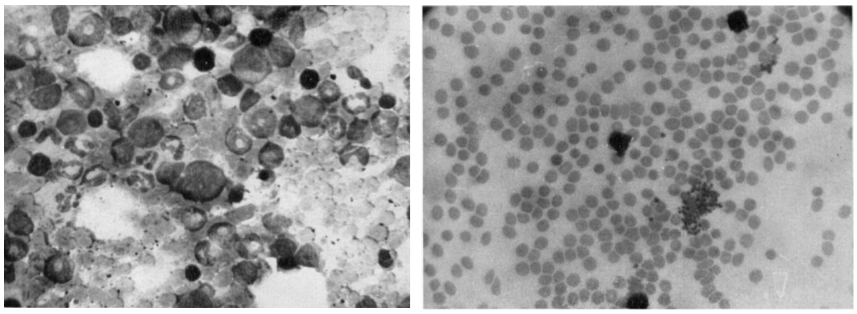


Figure 12.2. Bone marrow from a normal rat (left) and a rat treated with the active material purified from *Vinca rosea* (right). The bone marrow from treated rats (right) shows many red blood cells, but very few the larger developing white blood cells such as those seen in normal bone marrow on the left (Noble et al., 1958). *(Noble'Cutts'58annNYacad-vinca-chance-r)*

To give an idea of how they purified an active compound from extracts of the plant material, here is a brief example. The plant material is first soaked in a solvent, such as alcohol or ether. The material in the resulting solutions is then separated into different “fractions”. The researchers then injected samples of each fraction from various stages of purification into rats and monitored white blood cell counts in blood drawn daily from the rat's tail. Injecting the extract typically caused the rat's leukocyte count to drop after 2-3 days and then to recover about a week later. In the first step of purification, an acid extract (ethanol with 10% acetic acid) of the dried leaves was made alkaline, whereupon something precipitated that was more active than the original extract; thus the active ingredient had been partially purified. Further steps of purification eventually yielded a pure highly potent needle-like crystalline compound, which they named vincaleukoblastin, later shortened to vinblastine (Figure 12.3). The chemical structure of vinblastine, like that of many natural products, took a lot of work to unravel, because it is very complicated with many interlocking rings and asymmetric centers (Figure 12.4).

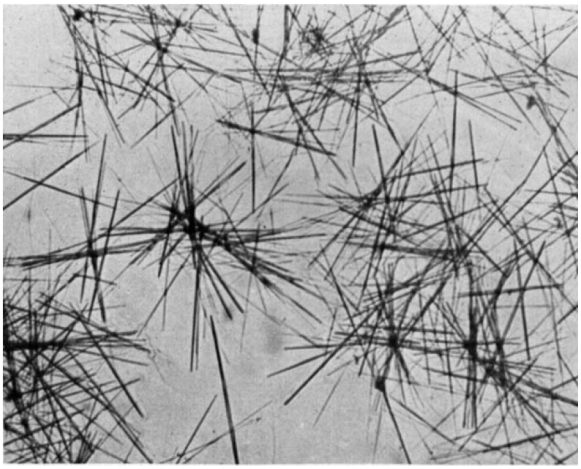


Figure 12.3. Needle-like crystals of vinblastine purified from the periwinkle *Vinca rosea* (Noble et al., 1958).

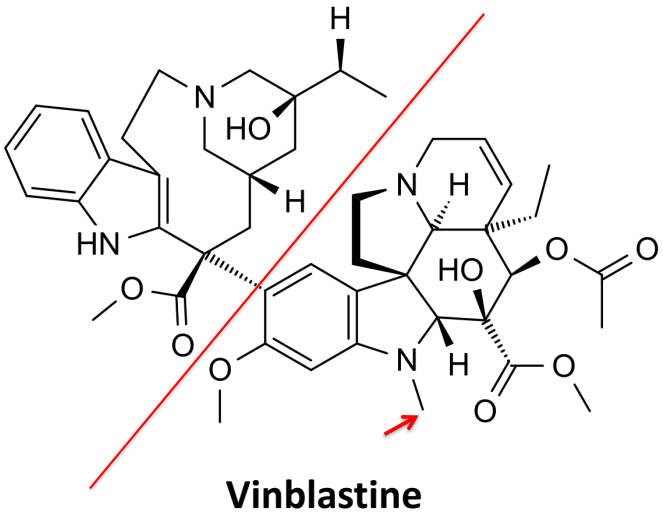


Figure 12.4. The vinblastine molecule consists of two parts: a "catharanthine nucleus" to the upper left of the red line and a "vindoline nucleus" to the lower right of the red line. Vincristine differs only in having an oxygen atom added at the red arrow (thus vincristine has a formyl group instead of a methyl group attached to the nitrogen at that position). The dashed bond that connects the 2 parts of the molecule indicates that the upper left half of the molecule is connected above the plane of the page, while the connection to lower right half is below the plane; the precise 3-dimensional geometry of the molecule is critical for activity: only the correct geometry works.

Even at this early stage on the road to vinca alkaloids as important anticancer drugs, the investigators already had clues to how the drug work and the drug's limiting toxicities. In addition to the blood count suppression, there was damage to the lining of the intestines. We now know that these drugs tend to kill dividing cells and that active cell division is required to maintain blood counts and to replenish cells that are continually being sloughed off from the intestinal lining. Blood count suppression and damage to the intestinal mucosa are two major toxicities of many anticancer drugs. Thus, the vinca story highlights the fact that attack on dividing cancer cells also impacts normal dividing cells and particularly normal tissues that critically rely on continual production of new cells. The damage to dividing cells in normal tissue is the main cause of dose-limiting toxicity by most anticancer agents.

(Gastrointestinal toxicity in the case of vinca alkaloids turned out not to be a major clinical problem, possibly because other toxicities supervened, and patients rarely received dosage high enough to cause troublesome gastrointestinal toxicity.)

In addition to vinblastine, several other alkaloids of related chemical structure were isolated, of which the most important, vincristine, differed from vinblastine only by addition of an oxygen atom at an important location in the molecule (Figure 12.4). Even though the change in chemical structure was tiny, the two drugs differed in the cancers they were most effective against. Most striking was the greater effectiveness of vincristine against acute leukemia (Johnson et al., 1963). Also, there was surprisingly little cross-resistance between the two vinca drugs; thus, when patients stopped responding to one of the vinca drugs, they sometimes subsequently responded to the other.

How vincristine and vinblastine block mitosis.

A major clue to how the two vinca's work soon emerged, when it was found that vinblastine blocked the cell division process. Moreover, the block was not at the DNA duplication stage, where most of the previously known anti-cancer drugs, such as methotrexate and 5-fluorouracil, block cells. Instead, the block was at mitosis, during which the chromosomes segregate into the two daughter cells. More precisely, the block was at the metaphase stage of mitosis, where the chromosomes are fully condensed and line up, ready to separate into their respective daughter cells (Figures 5 and 6) (Palmer et al., 1960) (Johnson et al., 1960) (Cutts, 1961). The block was due to the fact that the vinca drugs prevented the formation of the mitotic spindle microtubules. without which the chromosomes cannot separate, and the cells cannot divide.

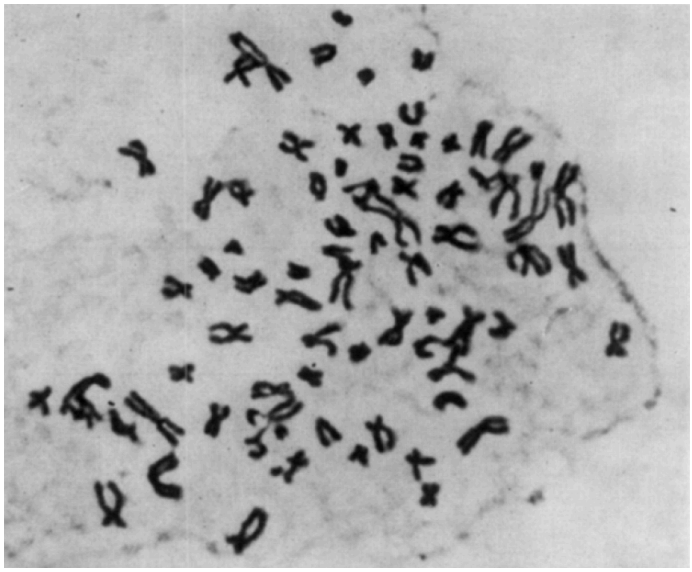


Figure 12.5. A vinblastine-treated cell arrested in a spindle-deficient metaphase. The chromosomes are condensed as in normal mitosis, except that the microtubules of the mitotic spindle is absent; therefore, the chromosomes are scattered instead of being lines up as they would be in a normal metaphase (Palmer et al., 1960).

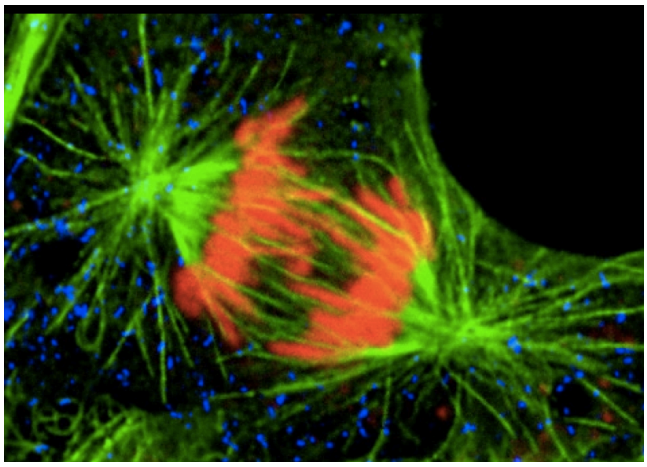


Figure 12.6. This beautiful picture shows a critical phase of a normal mitosis ("anaphase"), when the microtubules pull chromosomes into the two future daughter cells after a normal metaphase. The chromosomes are stained red, the microtubules, green. A vinblastine-treated cell lacks microtubules, and therefore cannot proceed from metaphase to anaphase.

Although the idea that mitotic block was plausible as the cause of cell killing by vinblastine, it was mere conjecture, because the drug has other actions as well. The

conjecture was soon supported by direct evidence: the extent of cell killing by various doses correlated quantitatively with the extent of microtubule inhibition; therefore the two effects were correlated and probably had related causality (Said and Tsimberidou, 2014; Tucker et al., 1977).

Further investigation revealed the molecular details of how the vinca alkaloids block mitosis. During mitosis, the chromosomes normally divide equally between the two daughter cells. Each chromosome set is pulled into its corresponding daughter cell through the action of the mitotic spindle (Figure 12.6). The mitotic spindle is made up of microtubules, which in turn are made up of tubulin molecules (Figure 12.7). That is where the vinca's attack: they bind to the tubulins and prevent them from assembling into microtubules. Instead of adding to the microtubules, the tubulins become bound by vincristine or vinblastine and aggregate into "paracrystalline" structures that are of no use to the cell (Na and Timasheff, 1982) (Figures 15.8 and 15.9).

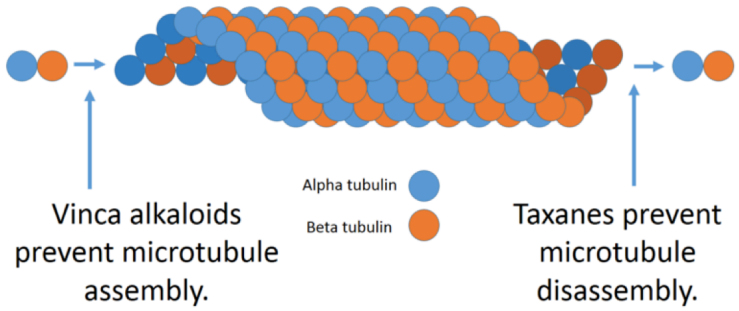


Figure 12.7. Microtubule are made up of alpha and beta tubulin units (blue and red). Vinca alkaloids were found to prevent tubulins from assembling into microtubules, whereas taxanes were found to lock the tubulins in place, so that the microtubules could not function. Either way, the progress of mitosis was blocked. Microtubule function requires that tubulin units be able to add at one end and be removed at the other end. The vincas made microtubules shrink and disappear, whereas the taxanes caused microtubules to accumulate in functionless bundles (Lobert et al., 1996).

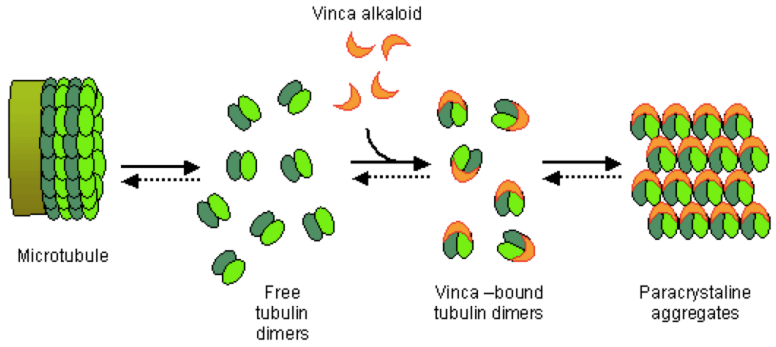


Figure 12.8. How vincristine or vinblastine sequester tubulin into useless paracrystalline aggregates. The end of a microtubule is depicted on the left. It consists of alpha- and beta-tubulin units (blue and green), which pair up. Vinca molecules (orange crescents) bind tubulin pairs (dimers) and assemble them into useless paracrystalline aggregates (right). (Source: Wikipedia.)

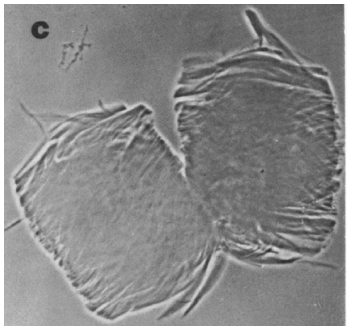


Figure 12.9. Paracrystalline bodies formed by vinblastin-bound tubulins (Na and Timasheff, 1982)

Vinblastine is effective against lymphomas.

In the early 1950's, Cutts had already noted that vinca extracts and the purified vinblastine -- the first vinca alkaloid to be isolated and tested -- inhibited the production of blood cells in the bone marrow of rats (Figure 12.2). He thought it might therefore work against leukemias, which are malignancies arising in the bone marrow. When given to leukemic mice, the drug indeed extended their lifespan considerably (Cutts et al., 1960; Johnson et al., 1960).

In view of the impressive activity of vinblastine in mice, clinical investigators at the Ontario Cancer Institute in Toronto, Canada administered the drug to patients with advanced stages of leukemia, lymphoma, or other malignant tumors (Warwick et al., 1960). At about the same time, a preliminary clinical trial of vinblastine was also

carried out at the Indiana University Medical Center (Hodes et al., 1960). In both studies, a few patients had partial remissions, but the results, although encouraging, were insufficient for firm conclusions. The main toxicity, as expected, was suppression of white blood cells (leukopenia). Vinblastine is still useful in the treatment of lymphomas such as Hodgkin's disease, but, for acute leukemia, it has been replaced by vincristine.

Vincristine is the star.

Vinblastine and vincristine differed in how effective they were against different malignant tumor, possibly due to differences in how much of the drug enters particular types of tissues (Zhou et al., 1990). The most striking difference however was the extraordinary effectiveness of vincristine against acute leukemia.

Vincristine differs from vinblastine only in the addition of an oxygen atom to an important part of the molecule (Figure 12.4), but it has become the more useful drug, especially in the treatment of acute leukemia. The first clinical study of vincristine for the treatment of acute leukemia in children was carried out at the National Cancer Institute by Myron R. Karon, Emil J Freireich, and Emil ("Tom") Frei (Karon et al., 1962). Karon unfortunately died of a cerebral hemorrhage in 1974 at the age of 42 at the height of his career as a leading researcher and pediatric oncologist {Hersh, 1975 #937}.

Frei and Freireich went on to lead the development of cancer chemotherapy and the cure of childhood leukemia. In the initial study, with Myron Karon, they escalated the vincristine dose slowly while closely monitoring the blood counts and bone marrow of their patients. When dangerous toxicity threatened, they lowered the dose of vincristine, and transfused whole blood, platelet-rich plasma, or leukocytes as needed. Of 12 children with acute lymphocytic leukemia who were treated with vincristine in that first study, 8 had a complete remission. This experience was the first indication that vincristine would become a major part of the cure of acute leukemia (Said and Tsimberidou, 2014).

But vincristine damages the nervous system.

Researchers were pleased that vincristine rarely produced serious toxicity to the blood-cell-forming bone marrow. However, they were not at all pleased that the amount of drug given to patients had to be limited to avoid damage to the nervous system (neurotoxicity) (Legha, 1986; Rosenthal and Kaufman, 1974).

Vincristine's neurotoxicity is first felt as numbness and tingling in the fingers and toes (Legha, 1986). The reason is simple. In addition to depleting the microtubules of the mitotic spindle, vincristine attacks the microtubules that fill the nerve cell's axon; the nerve cell needs those axonal microtubules to function and survive. The

axons long enough to reach the tips of fingers and toes contain the longest microtubules and therefore are most vulnerable to the drug. Vincristine causes the axons to degenerate and the nerve cells to die.

How can the neurotoxicity of vincristine be avoided?

Much effort was devoted to trying to reduce vincristine's neurotoxicity. At early stages of treatment, the ill-effects on peripheral nerves is reversible if the drug is discontinued; therefore, the dosage had to be kept within those limits. Several substances were investigated for possibly reducing this toxicity. Of those, glutamic acid received the greatest attention, and early studies were encouraging (Jackson et al., 1988). Recent studies unfortunately failed to confirm that hope (Bradfield et al., 2015).

In recent attempts to reduce toxicity in general and neurotoxicity in particular, vincristine has been incorporated into sub-microscopic fatty globules called liposomes (Raj et al., 2013). Toxicities may be less for the liposomal-vincristine, but the extent to which it reduced toxicity in relation to therapeutic efficacy remained uncertain.

Danger of inadvertent injection of vincristine into the spinal fluid.

Even though air travel has become very safe, a serious accident sometimes happens. Similarly, much care is required to eliminate serious medical mistakes due to human error. Since vincristine attacks the microtubules in nerve axons, one of the worst medical mistakes would be if the drug were accidentally injected into the spinal fluid. How could that happen? Here is what happened not long ago in Thailand to a 12-year old girl with acute lymphoblastic leukemia who was receiving treatment that would probably have cured her and saved her life (Chotsampancharoen et al., 2015). On the day of the error, she was to receive an intravenous injection of vincristine and a spinal injection of methotrexate as an essential part of the leukemia cure: methotrexate kills any leukemic cells in the central nervous system and does not damage normal brain cells (which rarely divide). Vincristine fortunately is kept out of the central nervous system by the "blood-brain barrier." Thus, vincristine acts safely against the leukemic cells outside of the central nervous system, Methotrexate is injected directly into the spinal fluid in order to kill any leukemic cells that lurk within the central nervous system. But it is disastrous if the two drugs were accidentally mixed up as to which is injected where – which is what happened in this tragic case. The injection kit that was provided for treatment of the leukemic child was provided with 2 syringes, each properly labeled for what it contained: vincristine or methotrexate. Somehow, the administering team mixed up the 2 syringes and used the vincristine syringe for the spinal injection. The team realized their mistake almost immediately and tried to flush the drug out of the spinal canal. Nevertheless, despite all efforts for supportive care, the child suffered

badly and died 5 days later. This case, as well as previous cases, were published with suggestions for additional safeguards to avoid such errors (Chotsampancharoen et al., 2015; Gilbar, 2012; Gilbar, 2014).

Taxol and the Pacific yew tree.

To find new anti-cancer drugs from nature, the National Cancer Institute began in 1960 under the direction of Jonathan L. Hartwell an ambitious program to collect natural products and screen them for their ability to kill cancer cells. If something kills cancer cells, however, it doesn't mean that it necessarily has anti-cancer activity, because the substance might kill normal cells just as well.

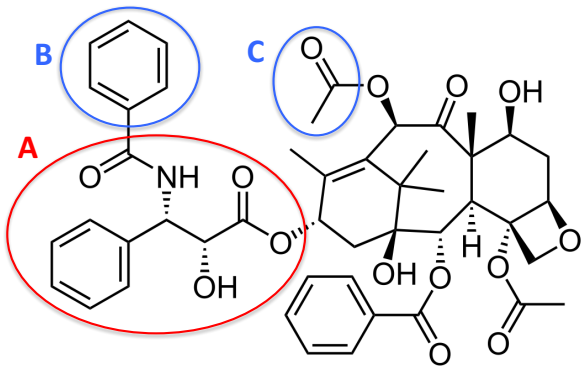
Some of the toxic extracts of plants or animals were selected to test whether they prolonged the life of tumor-bearing mice. The road from there to a useful anti-cancer drug however could be long and tortuous, and is well illustrated by the story of taxol, or "paclitaxel" as it is now called.

It is remarkable that three scientists who discovered taxol and initiated an understanding of how it worked also contributed similarly to the discovery of another major anticancer drug, camptothecin (Chapter 11): Monroe E. Wall, Mansukh C. Wani and Susan Band Horwitz (Figures 9.1 and 9.4). Wani and Horwitz described how they purified the drug and determined its chemical structure and mechanism of action (Wani and Horwitz, 2014). Other details of the story, including its political aspects, were published by Goodman and Walsch in *The Story of Taxol: Nature and Politics in the Pursuit of an Anti-Cancer Drug*, Cambridge University Press, 2001.

Figure 12.10. Left: The Pacific yew tree (*Taxus brevifolia*), the source of paclitaxel (Taxol). Right: Peeling the bark (image from the National Cancer Institute. Public domain.)

The Taxol story began in 1962, when Arthur S. Barklay, a botanist working for the U.S. Department of Agriculture (USDA) collected bark from a Pacific yew tree (*Taxus brevifolia*) in Washington State (Figure 12.10). The USDA had been commissioned by the National Cancer Institute to collect samples of plants from which extracts were to be prepared and tested for activity against a cancer cell line. Extracts of the yew bark indeed killed cancer cells in culture.

Although there was not enough material to test it adequately in tumor-bearing mice, a natural products chemist might be inclined to go after a biologically active compound even without knowing whether it may turn out be useful. Monroe E. Wall and his colleagues at Research Triangle Park in North Carolina however hoped that an anti-cancer agent was lurking within the bark extracts, and they had the skills, patience and determination to go after it. In 1964 they started a huge effort to carry this out. It required testing on cancer cells at all stages of a seemingly endless separation sequence. After 2-3 years of painstaking work, they had a purified material that prolonged the lives of cancer-bearing mice. They named the new drug "taxol," in honor of the genus name of the tree it came from, and by 1971 they had determined its chemical structure (Figure 12.11) (Wani et al., 1971). The drug was later renamed "paclitaxel" when Taxol became the brand name.



Paclitaxel (Taxol)

A, microtubule binding site
B, replaced by $(CH_3)_3CO-$ in docetaxel
C, removed in docetaxel

Figure 12.11. Structure of paclitaxel (Taxol) and the changes in docetaxel (Taxotere). The part of the molecule in the red circle (A), which is the same in paclitaxel and docetaxel, is where microtubules bind (de Weger et al., 2014). The blue circles show the parts of the paclitaxel molecule that were changed in going from paclitaxel to docetaxel. Removing the atoms in C leaves a hydrogen attached to the oxygen; that H

can dissociate, leaving a negative charge on the oxygen, which can be shared with the oxygen to the right. The resulting negative charge makes docetaxel more water-soluble than paclitaxel.

A glance at the complicated chemical structure of the taxanes (Figure 12.11) gives an appreciation of the difficulty of solving the structure. Not only does the molecule have a great many atoms, but the atoms are arranged in a complicated way with interlocking rings and many asymmetric centers (indicated by the thick and hatched bonds).

How the taxanes produce their anticancer effects.

Interest in taxol languished for several years because it was difficult to acquire enough bark material from which to isolate the drug, and because the drug's activity in leukemia test systems in mice was deemed mediocre (Wani and Horwitz, 2014). The chemical structure of taxol (Figure 12.11) was too complicated for chemists to synthesize routinely in the laboratory. However, interest in the drug mounted when taxol was found to work unusually well against a mouse melanoma called B16.

Susan Horwitz then made an important discovery. Working at the Albert Einstein College of Medicine in New York, she found that taxol blocked cells in mitosis by perturbing the function of the microtubules that make up the mitotic spindle; moreover, the way in which the drug affected microtubules was novel (Figure 12.7) (Schiff et al., 1979; Schiff and Horwitz, 1980).

(For many years, we had a small Taxol tree, acquired I think by our lab chief David Rall, growing in the hall of our laboratory building – 5th floor of building 37 on the NIH campus in Bethesda. The tree was located in the Northwest corner of the hall that went all around the exterior next to the windows, before the interior was rebuilt. That exterior hall had added cheer to our windowless labs and allowed a pleasant walk around as brief relief from long laboratory hours; some of us used to - gather to view the sunset and share our latest ideas. The redesign was in part fired by the misguided notion that the exterior hall was wasted space.)

Microtubules are composed of two types of subunits: the protein molecules alpha and beta-tubulin, which associate in pairs in a manner that produces alternating alpha-beta pairs in intact microtubules (Figures 15.7). Taxol binds to a specific site on beta-tubulin in the intact microtubule (Figure 12.12). The microtubules of the mitotic spindle are a framework on which various motor proteins apply forces to move the chromosomes appropriately during mitosis. It is a complicated process in which tubulin molecules are added to one end of a microtubule and removed from the other end. That is how a microtubule grows and shrinks and moves to its proper place in the spindle. Anyone brave enough to read all the details could find them in a comprehensive review article by Walczak and Heald (Walczak and Heald, 2008). More details can also be found in the early articles by Susan Horwitz and coworkers

(Schiff et al., 1979; Schiff and Horwitz, 1980) and in more recent review articles (Orr et al., 2003) and (Wani and Horwitz, 2014).

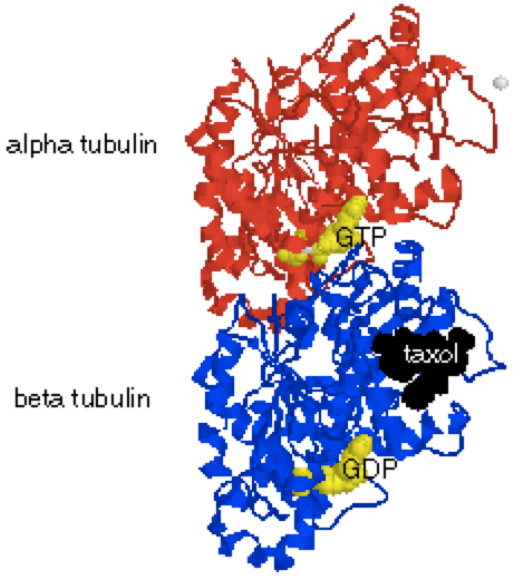


Figure 12.12. Structure of an alpha and beta tubulin pair (dimer) showing the backbones of those protein molecules. The place where Taxol binds, which is on the beta subunit, is shown in black. Red, alpha-tubulin; blue, beta-tubulin. Yellow, are GDP and GTP, which are small energy-bearing molecules that are essential for the structure and function of microtubules.

Therapy with taxanes.

Paclitaxel (as Taxol had by then been renamed) was becoming promising for treatment of several common cancers that were unresponsive or had developed resistance to other drugs. But two problems emerged that limited the dose levels that could be given to patients (Rowinsky and Donehower, 1995). First, was neurotoxicity: nerve damage, especially to the long nerves leading to the tips of fingers and toes. As already noted for vincristine, those long nerves are especially sensitive to anti-microtubule drugs, because long nerve axons contain long microtubules (Kudlowitz and Muggia, 2013) (Kudlowitz and Muggia, 2014). Those microtubules carry essential molecules from the cell body where they are made all the way to synapses at the end of axon.

The second problem was more specific to paclitaxel. Many patients developed hypersensitivity akin to the severe reactions that some people have to shellfish or beestings. The reactions to paclitaxel were sometimes even life-threatening. Here is

how that problem arose: Paclitaxel was so insoluble that an injectable preparation could not be made directly. Therefore, the drug was mixed with an oily substance called cremaphore, which is a chemical modification of castor oil. That solved the solubility problem, but created another problem: the hypersensitivity reactions that patients were experiencing turned out not to be due to paclitaxel itself, but to the cremaphore additive (Rowinsky and Donehower, 1995).

A new taxane: docetaxel.

The toxicity conundrum spurred the search for new paclitaxel-like drugs with hopefully less neurotoxicity and better solubility. A promising candidate was developed in 1981 from a compound extracted from another species of yew tree, the European yew *Taxus piccata* (de Weger et al., 2014) . The extracted compound of interest was itself inactive, but chemists had the insight to modify its structure in a way that conferred paclitaxel-like actions. The new drug, docetaxel (brand name Taxotere), which has a chemical structure similar to paclitaxel with two modifications (Figure 12.11), became mainstream in cancer treatment and research.

Clinicians did not give up on paclitaxel, however, because when one of the taxanes didn't work, sometimes the other did. The paclitaxel solubility problem was attacked in another way that turned out have other advantages. The drug was combined with albumin in aggregates of sub-microscopic clusters called nanoparticles (Henderson and Bhatia, 2007). Albumin is a blood protein having high solubility that also has the ability to bind many kinds of low-solubility molecules and distribute them through the blood stream. The new advantage was that these nanoparticles tend to leak out of blood vessels in tumors more easily than from blood vessels in normal tissues; therefore, the drug is somewhat selective for delivery into cancer tissues. Nab-paclitaxel, as the albumin conjugated form is called, was found to be clinically more effective than conventional paclitaxel (Henderson and Bhatia, 2007).

In retrospect, much has been accomplished in discovering and developing the taxanes that paralyze the microtubules required for cell division. As is the case with many other conventional anticancer drugs, the taxanes also attack dividing normal cells. Thus, paclitaxel and docetaxel, either as single agent or in combination with other drugs, sometimes slow the progression of some of the most common cancers. But, unfortunately the benefit is usually only to prolong life for a few months in a fraction of the cases. The tumors soon stop responding to the drugs, for example by acquiring mutations that lower the ability of the drugs to bind microtubules or to inhibit their function (Orr et al., 2003). Moreover, the quality of life during those few added months is often further degraded by the toxicity and side effects of the treatment.

Much has been learned about the taxanes and how they affect microtubules in cells whose function depends on them. But in the end the impact on the most common

cancers has thus far been pitifully meager. That might not be so surprising, considering that these materials evolved in nature for the purpose of chemical warfare among competing species of organisms. They nevertheless became more useful when combined with other drugs.

A marine sponge contributes new mitotic inhibitors: halichondrin B and eribulin.

In the quest for new and better anti-cancer drugs, the National Cancer Institute began collecting invertebrate marine animals and tested extracts for ability to kill cancer cells (Vindya et al., 2015). They hoped that some of the toxins made by those creatures in their natural biological warfare might be useful against cancers. One of the most promising finds came from a rare Japanese sponge called *Halichondria okadai* (Figure 12.13) (Hirata and lJemura, 1986; Swami et al., 2015). Extracts from this organism were extraordinarily potent in killing cancer cells in culture, and the active component became a new anticancer drug, halichondrin B. The isolation of this rare molecule and the determination of its complicated chemical structure were themselves a *tour de force* (Figures 15.14). But, coupled with the novel way in which its mechanism of action was unraveled (to be explained below), makes this a truly remarkable achievement. The NCI team credited with this work is depicted in Figure 12.15.

Chemists then isolated the most active toxin, which they named halichondrin B, and determined its complicated chemical structure (Figure 12.14). The drug held promise, because it suppressed several human tumors transplanted into immune-deficient mice ("xenograft tumors") (Fodstad et al., 1996). Further progress was hampered however, because it was difficult to obtain enough material from that rare sponge, and the chemical structure was too complex to prepare routinely in the laboratory. Therefore, chemists prepared simpler structures by leaving out parts of the full Halichondrin B molecule, hoping to hit upon a compound that had the desired activity and that was feasible to synthesize in sufficient quantity. That effort yielded a promising new anti-cancer drug: eribulin (Figure 12.14) (Dybdal-Hargreaves et al., 2015) (Thara and Gitlitz, 2014). The chemists could be congratulated for having the insight that allowed them to select a small part of the halichondrin molecule that was active and that they could synthesize in the laboratory. Moreover, the new synthetic drug, eribulin, had better solubility than the parent halichondrin.

Halichondria

Figure 12.13. Halichondria, the type of marine sponge from which halichondrin B was extracted. (From Wikipedia.)

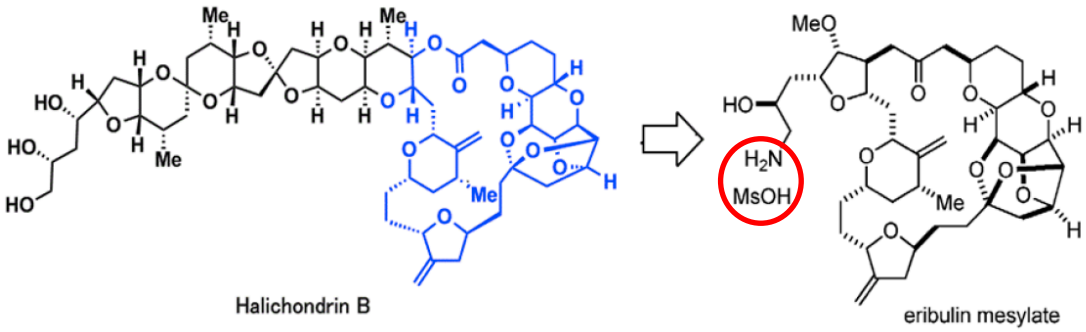


Figure 12.14. Chemical structures of the natural product Halichondrin B (left) and its synthetic derivative Eribulin (right). The latter has a positive charge on the NH2 group, which is paired with the negatively charged mesylate (MsOH) ion (red circle). The additional charged group makes eribulin more soluble than halichondrin. The part of the halichondrin B molecule that is preserved in eribulin is shown in blue. ((Swami et al., 2015). It was a pleasant surprise that such a small part of the halichondrin molecule retained activity.

Figure 12.15. The National Cancer Institute's halichondrin team in 1992. From left to right: Robert Shoemaker, Ernest Hamel, George Pettit, Kenneth Paull, Michael Boyd (Shoemaker, 2006). (All were NCI staff, except for George Pettit who was Professor of Chemistry at Arizona State University and worked under NCI contract.)

How Halichondrin B was found to be a microtubule inhibitor.

That halichondrin was a mitotic inhibitor was first indicated by cell toxicity assays in the National Cancer Institute's 60 human cell lines (NCI-60) (Figure 12.16). The pattern of toxicity (inhibition of cell growth and/or increase in cell killing) among the cell lines showed that halichondrin B had similar effects to those of maytansine, a known microtubule inhibitor. This mechanism of action was confirmed by Earnest Hamel at the NCI, who showed that halichondrin B binds tubulin and inhibits its assembly into microtubules in a manner similar to vinca alkaloids (Bai et al., 1991).

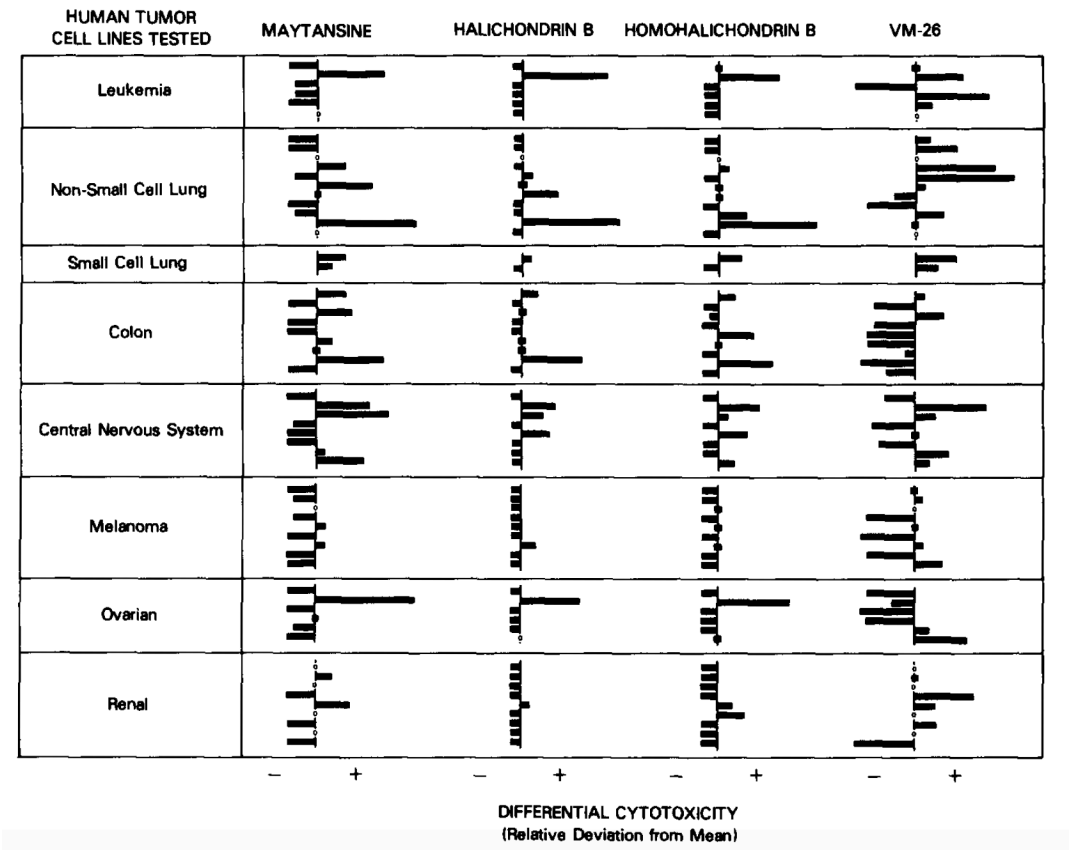


Figure 12.16.: Toxicity patterns (inhibitions of cell growth and/or increase in cell killing) in the National Cancer Institute's 60 cell line panel (NCI-60). The pattern for halichondrin (2nd from the left) resembled that of maytansine (a known microtubule-targeted drug) (left-most) and differed from the pattern of VM26 (teniposide, a topoisomerase II targeted drug) (Bai et al., 1991). This was the first clue that halichondrin targets mitotic microtubules. This method of comparing anticancer cell activities was developed by NCI's Kenneth Paull.

Other mitotic inhibitors: prospects and surprises

Many other microtubule-targeted inhibitors have been isolated from plants and animals or made in the laboratory by chemists who continue to modify their structures in hope of finding new useful drugs (Jiang et al., 2006; Jordan et al., 1998). Several have been tested in early clinical trials (phase 1 or 2), but none of them have, as of this writing, become part of our anti-cancer armamentarium.

Aside from microtubules, there are other essential mitotic spindle components that are being investigated as potential anti-cancer targets (Jiang et al., 2006). Although none have yet been demonstrated to have useful anti-cancer action, we can briefly state what those targets are without going into detail: inhibitors have been developed to target (1) kinesin motor proteins that move chromosomes during mitosis by attaching to and pulling on spindle microtubules, (2) aurora kinases that

are required to initiate the formation of the mitotic spindle, and (3) polo-like kinases that are required to turn on the machinery that initiates entry of the cell into mitosis. This gives an idea of the extensive terrain remaining for researchers to explore for new mitotic inhibitors.

A small change converts a microtubule blocker into a topoisomerase blocker.

(Krishan et al., 1975)

Podophyllin has a long history of medicinal use by Native Americans, and it was used as a suicide agent by the Iroquois (Kelly and Hartwell, 1954). In recent times, it became the source of two very different types drugs, although, remarkably, both types derived from the same chemical structure. First, podophyllin (podophyllotxin) was found to be a mitotic inhibitor like colchicine and vinblastine. A small change in chemical structure however yielded the important anti-cancer epipodophyllotoxins etoposide and teniposide, which work in an entirely different way: they target topoisomerase II (Chapter 10).

Podophyllin resin is made from the mayapple, also known as the American mandrake. The active podophyllotoxin is in the plant's roots, leaves, and creeping underground rhizomes, which were used by Native Americans as an emetic and cathartic and to expel parasitic worms from the intestinal tract (Small and Catling, *Canadian Medicinal Crops*, NRC Research Press 1999, cited by Wikipedia). The first U.S. Pharmacopeia (1820) listed podophyllin as a cathartic but was dropped in the 12th revision (1942). Interest revived when it as found an effective dermatologic treatment of condyloma acuminata (genital warts now known to be caused by certain human papilloma viruses (HPV)) (Kelly and Hartwell, 1954; King and Sullivan, 1946).

Investigating why podophyllin was effective against genital warts, King and Sullivan (King and Sullivan, 1946) applied it to the skin of rabbits and noted unusual changes in the nuclei of the skin cells. They thought that many of the cells were in a distorted state of mitosis, similar to that produced by colchicine. These simple observations, reported in a brief note in *Science* in 1946, already led the researchers to a correct idea about what the drug does to cells.

In a subsequent note in *Science*, Sullivan and Wechsler (Sullivan and Wechsler, 1947) looked at the effects of podophyllin in onion root tips, a convenient tissue for study of mitosis in the rapidly proliferating cells of the growing root. They confirmed the colchicine-like block of mitosis and thought podophyllin useful for cell division studies; they noted that "podophyllin is readily available at pharmaceutical supply houses and may be obtained at approximately 90 cents for four ounces."

Podophyllin was tested in a variety of experimental systems, including tumors in mice, and clinically, especially in treatment of various skin conditions, but the main lasting clinical application has been for genital warts. The extensive history of podophyllin studies and trials was compiled by Margaret Kelly and Jonathan Hartwell in the NCI's former Laboratory of Chemical Pharmacology (Kelly and Hartwell, 1954).

Mitotic inhibitors: overview

The unusual sources and chemistry of the major mitotic inhibitor drugs may at first be puzzling. Unlike DNA damaging agents, they do not have highly reactive (covalent bond-forming) chemistries. Unlike the DNA synthesis inhibitors, they are not analogs of vitamins or molecules of the cell's normal biochemistry. They are not antibiotics such as are produced by microorganisms. Instead, they are complicated molecules that do not at all resemble any of the cell's normal molecules. Also, they derive almost exclusively from plants or animals, including marine invertebrates. They almost all come from creatures whose cells have a nucleus ("eukaryotes") that undergoes mitosis; in other words, mitotic inhibitors are made almost exclusively by organisms that engage in mitosis. They seem to be poisons used in the competition (biological warfare, if you will) between eukaryotes in nature.

This chapter was about 3 classes of mitotic inhibitors: vinca alkaloids, taxanes, and halichondrins that have established roles in cancer chemotherapy. However there are other classes of mitotic inhibitors, most of them from eukaryotic animals or plants (Jordan et al., 1998). Several are or have been in clinical development; some have been discarded as ineffective or too toxic, but several remain promising and are still being studied.

Mitotic inhibitors bind and disable the microtubules whose function is required for cell division. But microtubules also have other important functions in the cell, functions that do not involve cell division. Inhibition of mitosis however seems to be the major anti-cancer action of these drugs. We have seen that some of these drugs disrupt microtubules in the axons of nerve cells, resulting in sometimes severe neurotoxicity.

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